BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PATENT SPECIFICATION (11) 1 579 490

1 579 490

(21) Application No. 21588/77 (22) Filed 23 May 1977 (19)

(31) Convention Application No. 7615734 (32) Filed 25 May 1976 in

(33) France (FR)

(44) Complete Specification Published 19 Nov. 1980

(51) INT. CL.[3] C08J 9/26
A61K 31/74

(52) Index at Acceptance
C3C 129 180 181 358 388 452 454 545 548 549
A5B 180 350 35Y 380 38Y 39X H
C3Y B120 B121

(54) MICROPOROUS HYPOCHOLESTEROLEMISING RESIN

(71) We, ESTABLISSEMENT VIRIDIS, a body corporate organised and existing under the laws of Liechtenstein, of 9490 Vaduz, Liechtenstein, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

This invention relates to a new microporous ion exchange resin for use in human therapy as a hypocholesterolemising agent. Ion exchange resins notably find use in the treatment of various pathological states such as hyperacidity, prevention of Na^+ depletion in the gastroenteric tract, induction of K^+ depletion, nephrotic, pancreatic and cardiac treatment, ulcer treatment, neutralisation of gastric acidity etc.

Obviously each particular pathological state requires a resin of particular chemical characteristics, chosen from the group consisting of weakly acidic resins, strongly acidic resins, weakly basic resins, and strongly basic resins. A fundamental requirement being that the resins are free from toxicity towards the human organism.

The use of ion exchange resins has notably been extended in recent years to the treatment of hyperlipidemias.

Premature arteriosclerosis can develop in the organism with excessive levels of liquids, primarily cholesterol, with consequences such as cardiac infarction and cerebral thrombosis. It is therefore a problem of large dimensions for which the resolutive drug has not yet been found.

To reduce the cholesterol to normal levels, it is necessary to act in two ways, (1) by excluding all foods rich in cholesterol or saturated fats, and (2) by increasing the elimination of the cholesterol. Basic ion exchange resins act in this second manner by fixing the bile acids at the intestinal level, and thus interrupting their entero-hepatic recycle with consequent loss of cholesterol.

The basic ammonium and amino resins used up to the present time (essentially Cholestiramine - British Patent No. 929,391) have a maximum exchange capacity of around 3.5 meg/g, a value which is too low for a very good result.

On the other hand, it is not possible to increase the content of basic groups in the resin and therefore its exchange power cannot be increased beyond said limit.

It has also been proposed (British Patent No. 1,286,949) to use ammonium or amino resins comprising a macroporous structure instead of a compact structure of the Cholestiramine type.

However these resins have never been used in practice, the results initially obtained in vitro have not been confirmed in vivo because the methods of determination which were used experimentally were not suitable to test their capacity for stably adsorbing the cholesterol and bile acids.

In reality, the quantities of bile acids fixed in the effective times and at the effective pH values of the digestive tube for the macroporous resins are less than those obtained with the compact resins. Even the values obtained for fixing triglycerides have not been confirmed in vivo, and these resins have proved overall inferior to Cholestiramine in the plasmatic cholesterol dimension.

We have now discovered a new type of ion exchange resin specifically suitable for fixing the bile acids and forming the object of the present invention, and which by exploiting not only the chemical properties but also the physical structure attains a much higher degree of exchange power and returns the altered cholesterol levels to normal values.

Accordingly, the invention provides a microporous anionic resin having a cross-linked polystyrene matrix comprising active moieties selected from amino; dialkylamino or trialkylammonium; said microporous, anionic resin having the following characteristics:

percentage of cross-linking	:	8-20%
exchange capacity	:	3.9-6 meq/g
porosity	:	0.4-0.6 cc/g
average pore diameter	:	150-200 Å
specific surface area	:	70-100 m^2/g
real density	:	0.9-1.12 g/cc
apparent density	:	0.5-0.6 g/cc
particle size distribution	:	60-80 mesh

The difference in values between the real and apparent densities is due to the pore (or void) volume. The real density is the density of the solid part of the resin whilst the apparent density is the density of the resin including the pores. The difference between the two values is a measure of the porosity of the resin. The mesh is U.S. Standard mesh. The term "percentage of cross-linking" means the quantity, by weight, of the cross-linking monomer to the total weight of the monomer mixture.

When the active moiety is trimethylammonium the resin preferably has the following characteristics:

percentage of cross linking	:	11.3%
exchange capacity	:	3.9 meq/g
porosity	:	0.4 cc/g
average pore diameter	:	200 Å
specific surface area	:	70 m^2/g
real density	:	1.10 g/cc
apparent density	:	0.54 g/cc
particle size distribution	:	60-80 mesh

When the active moiety is dimethylamino the resin has the preferred characteristics:

percentage of cross linking	:	11.3
exchange capacity	:	5.7 meq/g
average pore diameter	:	200 Å
specific surface area	:	85 m^2/g
porosity	:	0.6 cc/g
real density	:	1.12 g/cc
apparent density	:	0.50 g/cc
particle size distribution	:	60-80 mesh

The invention also provides a method of preparing a microporous resin comprising:

(a) treating styrene with 8-20% by weight of a cross-linking monomer in the presence of 80-150% by weight of the total monomer weight of a porosity agent and 0.5-2% by weight of a catalyst initiator to afford copolymerized styrene resin and

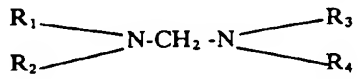
(b) introducing into said resin of step (a) amino, dialkylamino or quaternary ammonium moieties; wherein said porosity agent is squalene or a hydrogenated steroid having a molecular weight of 200-500.

Preferably said cross-linking monomer is selected from divinylbenzene, divinyltoluene,

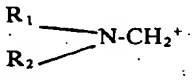
divinylnaphthalene divinylxylene or divinylethylbenzene; said porosity agent is squalene;

said step (b) comprises treating said resin of step (a) with chloromethylmethyl ether or bis-chloromethyl ether, in the presence of a Lewis acid catalyst, to afford a chloromethyl substituted styrene resin; and then treating said chloromethyl substituted styrene with ammonia, dialkylamine or trialkylamine.

The most preferred cross-linking monomer is divinylbenzene. Step (b) may also be carried out by reacting the resin formed in step (a) with diamines of the type



in which R_1, R_2, R_3 and R_4 which may be the same or different are alkyl groups of 1 to 4 carbon atoms. Under suitable conditions (temperature of around 100°C and pressure of approximately 3 atms) these diamines liberate the ion:



which reacts with the free hydrogen atoms in the styrene rings. The methyl-amino groups thus bonded to the polymer matrix may be further alkylated to achieve the maximum degree of nitrogen substitution (quaternary nitrogen) with common alkylating agents such as dimethyl sulphate, methylene chloride, bromide or iodide.

Preferably the catalyst for initiating the polymerization is lauroyl peroxide, benzoyl peroxide tertiary butyl peroxide, cumene peroxide or acetyl peroxide.

The polymerization may be carried out in suspension in a medium in which the monomers are insoluble. A suitable medium is water in the presence of a dispersing agent such as the ammonium salt of a styrene-maleic anhydride copolymer, carboxymethylcellulose or bentonite.

The preferred porosity agent is squalene in a 1:1 weight ratio relative to the monomer mixture. Any excess porosity agent may be removed, after the polymerization step, by an organic solvent such as hot ethyl alcohol.

The Lewis acids used in the chloromethylation step (step (2)) are preferably $AlCl_3$, $ZnCl_2$, $TiCl_4$, $TiCl_3$, $SnCl_4$, $FeCl_3$.

The following examples illustrate the invention.

EXAMPLE I

A mixture of 33.3 parts by weight of styrene, 16.7 parts of divinylbenzene (DVB) with a titre of 60%, and 50 parts by weight of squalene is suspended, with stirring, in a 20% by weight aqueous solution of gelatine. One part of bentonite is added to the suspension, and 0.2 parts by weight of lauroyl peroxide as catalyst. The suspension is heated for 40 hours at 65°C and then for 10 hours at 90°C. The opaque pearls thus obtained are carefully washed. The squalene is then extracted with ethyl alcohol in soxelet. The extraction is followed by stripping with steam and then drying in an air current.

Part of the dry product is treated with five parts of chloromethylmethylether and two parts of zinc chloride at a temperature of 50°C for seven hours to obtain a product with a 19% Cl content.

The mixture is then pured into 20 parts of H_2O and neutralised with a concentrated aqueous NaOH solution.

The chloromethylated intermediate is dried, partially swollen with toluene (22% by weight) and treated with 3 parts of a 70% aqueous solution of trimethylamine at a temperature of 80°C in an autoclave under pressure for five hours. The product is then washed first with water and then with a 5% NaCl solution.

A microporous anionic exchange resin is obtained with the following characteristics:

- percentage of cross-linking 11.3
- exchange capacity 3.9 meq/g
- average pore diameter approximately 200 Å
- specific surface area 70 m^2/g
- porosity 0.4 cc/g
- real density 1.10 g/cc
- apparent density 0.54 g/cc
- particle size distribution 60-80 mesh

EXAMPLE II

The polymerisation was conducted as in example I but using a monomer/porosity agent ratio of 1.2:1 and a porosity agent consisting of 70 parts of squalene, 20 parts of n-octanol and 10 parts of paraffin (41-45°C), to give a microporous resin of the following characteristics:

- percentage of cross-linking 11.3
- exchange capacity 4.5 meq/g
- average pore diameter approximately 190 Å
- specific surface 85 m^2/g
- porosity 0.6 cc/g
- real density 1.11 g/cc
- apparent density 0.50 g/cc
- particle size distribution 60-80 mesh

EXAMPLE III

A polystyrene matrix is prepared exactly as described in example II. However in the chloromethylation stage the catalyst used is $FeCl_3$ instead of $ZnCl_2$, but in the same ratio by weight. A chloromethylated intermediate is obtained containing 25% of Cl. This intermediate is aminated with a quantity of dimethylamine (60% solution) equal to 60% by weight of the chloromethylated product in the presence of NaOH (100%) at a temperature of 140°C and a pressure of 10 atm for three hours.

A microporous resin is obtained having the following characteristics:

- percentage of cross-linking 11.3
- exchange capacity 5.7 meq/g
- average pore diameter approximately 200 Å
- specific surface area 85 m^2/g
- porosity 0.6 cc/g
- real density 1.12 g/cc
- apparent density 0.50 g/cc
- particle size distribution 60-80 mesh

To demonstrate the capacity of the new resins according to the invention to eliminate the bile acids present in the intestine and thus to interrupt the liquid entero-hepatic cycle, some tests were carried out in vitro and in vivo and compared with a known resin (Cholestiramine) and a macroporous resin, namely "Lewatit" (Registered Trade Mark) MP-500. The results are summarised below.

The resins used were prepared in accordance with example II and example III, and identified respectively by the symbols SSC_1 and SSC_2.

1. *Absorption in vitro of sodium cholate as a function of the resin quantity.*

Glass containers are prepared each containing 20 ml of Na cholate at a concentration of 2 mg/ml in a 0.02 M phosphate buffer (pH6). 1 ml of H_2O and increasing concentrations of the resins are added to each container.

After stirring at 25° for three minutes, the container content is filtered and the cholic acid of the filtrate is determined in total mg using the spectrophotometry method after reaction with sulphuric acid. (Kier et al., J. Clin. Invest., 40,755,1952). Table 1 shows the results obtained as an average of 10 tests carried out for each resin.

TABLE 1

From the values given, it is apparent that the new SSC_1 and SSC_2 resins are able to fix more sodium cholate in vitro than Cholestiramine for equal dry weights, and that the macroporous resins are much worse than Cholestiramine.

Table 1

mg dry resin	20	40	60	80	100
Cholestiramine	19 ± 0.8	12 ± 0.6	8 ± 0.2	6 ± 0.2	4 ± 0.8
SSC_1	12 ± 0.4**	4 ± 0.5**	ND	ND	ND
SSC_2	11 ± 0.3*	8 ± 0.2*	1 ± 0.2*	ND	ND
"Lewatit" MP 500	25 ± 0.8	23 ± 0.7	20 ± 0.5	17 ± 0.5	15 ± 0.5

ND = undeterminable cholic acid

* $P < 0.05$ versus Cholestiramine

** $P < 0.01$ versus Cholestiramine

2. *Absorption in vitro of sodium cholate as a function of the incubation time.*

Glass containers were prepared each containing 10 ml of 0.02 M phosphate buffer of pH 6, 40 mg of sodium cholate and 20 mg of dry resin. The quantity of sodium cholate was determined at the times indicated using the spectrophotometry method of the previous paragraph 1.

Table 2

	Minutes			
	3	5	10	20
Cholestiramine	19 ± 0.8	18 ± 0.8	6 ± 0.2	4 ± 0.2
"Lewatit" MP 500	25 ± 0.8	22 ± 0.8	16 ± 0.5	10 ± 0.3
SSC_1	12 ± 0.4	4 ± 0.5	2 ± 0.3	ND
SSC_2	11 ± 0.3	5 ± 0.4	3 ± 0.4	1 ± 0.2

These tests clearly show that the resins according to the invention have a much greater speed of cholic acid fixing than Cholestiramine or macroporous resins.

This information is very important for the practical use of the resins in relation to the effective contact times with the bile acids in the organism.

3. *Absorption in vitro of sodium cholate as a function of the pH.*

The pH range considered is that range which is effectively present in the digestive tract.

Glass containers were prepared each containing 40 mg of sodium cholate, 10 ml of 0.02 M phosphate buffer and 20 mg of dry resin. The incubation time was 10 minutes. The method for determining the cholic acid content was that indicated in previous paragraphs 1 and 2.

Table 3

	pH			
	5	6	7	8
Cholestiramine	5 ± 0.3	6 ± 0.2	8 ± 0.4	9 ± 0.5
"Lewatit" MP 500	15 ± 0.5	16 ± 0.5	18 ± 0.8	19 ± 0.6
SSC_1	2 ± 0.3	2 ± 0.3	3 ± 0.4	4 ± 0.4
SSC_2	2 ± 0.5	3 ± 0.4	3 ± 0.4	4 ± 0.4

The data obtained again indicates clearly the superiority of the new resins relative to Cholestiramine and chemically analogous macroporous resins.

4. *Elimination in vivo of sodium cholate.*

Male rats of Wister stock weighing 150 g each were put on a standard diet containing 1% of cholesterol and 0.5% of sodium cholate.

The rats were divided into four groups of five each.

In the first group the rats received only the standard diet. In the second group they also received Cholestiramine resin, in the third group SSC_1 resin and in the fourth group SSC_2 resin.

All the resins used were administered orally in a dose of 800 mg/kg in two administrations per day.

After seven days from the beginning of treatment, a solution of Na cholate dissolved in a phosphate buffer containing 10 mg of cholate and 0.1 μC of C^{14} Na cholate was administered to the rats by gastric probe each evening for three days.

The rats were transferred into individual metabolic cages and the feces were collected for radioactivity counts for three days from the beginning of the treatment with C^{14} Na cholate.

The fecal radioactivity was monitored daily and the results expressed as a percentage of the controls.

With the Cholestiramine, on the first day there was an increase of 80% in the radioactivity present relative to the controls.

The percentage increase was 84% and 87% on the two successive days. With SSC_1, the increase in radioactivity of the excretions on the three days was 126, 144 and 163%.

With SSC_2, these values were 138, 151, 149%. A statistical analysis of the data shows that these differences in the two resins relative to Cholestiramine are highly significant.

5. *Elimination in vivo of cholesterol in rabbits.*

Male adult rabbits of New Zealand stock were used. The rabbits were divided into five groups each containing 20 rabbits, namely (1) controls, (2) rabbits treated with 1 g/day of cholesterol suspended in arachis oil, (3) rabbits treated with 1 g/day of cholesterol plus 0.5 g/kg/day of Cholestiramine, (4) rabbits treated with 1 g/day of cholesterol plus 0.25 g/kg/day of SSC_1 resins, (5) rabbits treated with 1 g/day of cholesterol plus 0.5 g/kg/day of "Lewatit" MP 500.

The rabbits were fed a normal diet and treated with cholesterol using a gastric probe. A 10% suspension of gum arabic was administered to rabbits of group (3), (4) and (5) sixty minutes before the cholesterol.

After 31 days the rabbits were sacrificed and the total amount of cholesterol in the blood was determined.

The results obtained demonstrate clearly that in vivo the macroporous resins are almost inactive, whereas the new resins according to the invention have an activity much greater than Cholestiramine.

Table 4

Group		Total plasmatic cholesterol, mg %
(1) -	Controls	82 ± 6
(2) -	1g/day cholesterol	713 ± 53
(3) -	1g/day cholesterol + 0.50 g/kg/day Cholestiramine	** 160 ± 12 *
(4) -	1g/day cholesterol + 0.25 g/kg/day SSC_1	** 108 ± 5 *
(5) -	1g/day cholesterol + 0.50 g/kg/day Lewatit MP 500	511 ± 28 *

* $P < 0.05$ versus 2

** $P < 0.05$ versus 5

6. *Elimination in vivo of cholesterol in rats.*

Adult male rats of the Sprague-Dowley stock were used and were fed with a Nath diet (J. Nutrit. 67,289, 1953).

The rats were divided into five groups each containing 20 rats, namely (1) controls, (2) rats fed with the Nath diet, (3) rats fed with the same diet + 0.5 g/kg/day of Cholestiramine, (4) rats fed with the same diet plus 0.25 g/kg/day of SSC_1, (5) rats fed with the same diet plus 0.5 g/kg/day of "Lewatit" MP 500.

After 30 days the rats were sacrificed and the total amount of cholesterol in the blood was determined. The results, contained in the following table, show that again in this case the macroporous resins are practically inactive in vivo, and the resins according to the invention have an activity much greater than Cholestiramine.

Table 5

		Total plasmatic cholesterol, mg%
1 -	Controls	100.4 ± 5
2 -	Nath diet	260.9 ± 13
3 -	D.N. + 0.5 g/kg/day Cholestiramine	** 120.1 ± 4 *
4 -	D.N. + 0.25 g/kg/day SSC_1	** 100.5 ± 7 *
5 -	D.N. + 0.5 g/kg/day Lewatit MP 500	210.2 ± 9 *

* $P < 0.05$ versus 2

** $P < 0.05$ versus 5

Toxicity tests conducted on the resins prepared in accordance with examples I, II and III indicated that the LD_{50} was not determinable. Clinically conducted tests confirmed the results obtained in the laboratory tests, and in fact the superiority of the new resins relative to Cholestiramine was more marked.

Therapeutic doses lie between 8 and 20g/day with an administration of 2 - 7 g one hour before meals.

WHAT WE CLAIM IS:

1. A microporous anionic resin having a cross-linked polystyrene matrix comprising active moieties selected from amino; dialkylamino or trialkylammonium; said microporous, anionic resin having the following characteristics:

percentage of cross-linking	:	8-20%	
exchange capacity	:	3.9-6 meq/g	
porosity	:	0.4-0.6 cc/g	
average pore diameter	:	150-200 Å	
specific surface area	:	70-100 m^2/g	
real density	:	0.9-1.12 g/cc	
apparent density	:	0.5-0.6 g/cc	
particle size distribution	:	60-80 mesh	

2. A resin according to claim 1 wherein the active moieties are trimethylammonium and has the following characteristics:

percentage of cross-linking	:	11.3%
exchange capacity	:	3.9 meq/g
porosity	:	0.4 cc/g
average pore diameter	:	200 Å
specific surface area	:	70 m^2/g
real density	:	1.10 g/cc
apparent density	:	0.54 g/cc
particle size distribution	:	60-80 mesh

3. A resin according to claim 1 wherein the active moities are dimethylamino and has the following characteristics:

percentage of cross-linking	:	11.3
exchange capacity	:	5.7 meq/g
average pore diameter	:	200 Å
specific surface area	:	85 m^2/g
porosity	:	0.6 cc/g
real density	:	1.12 g/cc
apparent density	:	0.50 g/cc
particle size distribution	:	60-80 mesh

4. A method of preparing a microporous anionic resin comprising:

(a) treating styrene with 8-20% by weight of a cross-linking monomer in the presence of 80-150% by weight of a catalyst initiator to afford copolymerized styrene resin and

(b) introducing into said resin of step (a) amino, dialkylamino or quaternary ammonium moieties, wherein said porosity agent is squalene or a hydrogenated steroid having a molecular weight of 200-500.

5. A method according to claim 4 whercin said cross-linking monomer is selected from divinylbenzene, divinyltoluene, divinylnaphthalene, divinylxylene or divinylethylbenzene; said porosity agent is squalene;

said step (b) comprises treating said resin of step (a) with chloromethylmethyl etheror bis-chloromethyl ether, in the presence of a Lewis acid catalyst, to afford a chloromethyl substituted styrene resin; and then treating said Chloromethyl substituted styrene resin with ammonia, dialkylamine or trialkylamine.

6. A method according to claim 4 or 5 wherein said cross-linking monomer is divinylbenzene.

7. A method according to claim 4 or 5 whercin said catalyst initiator is lauroyl peroxide,

benzoylperoxide, tertiary butyl peroxide, cumene peroxide or acetyl peroxide.

8. A method according to claim 4 or 5 wherein said polymerisation is carried out in a medium in which said monomers are insoluble.

9. A method according to claim 5, wherein said Lewis acid is $AlCl_3$, $ZnCl_2$, $TiCl_4$, $TiCl_3$, $SnCl_4$, $FeCl_3$.

10. A method according to any one of claims 4 to 9 wherein said porosity agent is squalene in a 1:1 weight ratio with said cross-linking monomer.

11. A method according to claim 4 further comprising removing any excess porosity agent by extraction with an organic solvent.

12. A method according to claims 4, 8 and 11 comprising:

(1) treating styrene with divinylbenzene in the presence of squalene in an aqueous solution of gelatin and bentonite; adding lauroyl peroxide as a catalyst and extracting residual squalene with ethyl alcohol to afford a copolymerized resin;

(2) treating the resin of step (1) with chloromethylmethyl ether and zinc chloride to afford the corresponding chloromethyl intermediate; and

(3) subjecting the intermediate of step (2) to treatment with trimethylamine to afford a microporous, anionic resin.

13. A therapeutic composition having hypocholesterolemising activity which comprises as the active ingredient an anionic microporous resin having a cross-linked polystyrene matrix comprising active moieties selected from amino, dialkylamino or trialkylammonium; said resin having the following characteristics:

percentage of cross linking	:	8-20%
exchange capacity	:	3.9-6 meq/g
porosity	:	0.4-0.6 cc/g
average pore diameter	:	150-200 Å
specific surface area	:	70-100 m^2/g
real density	:	0.9-1.12 g/cc
apparent density	:	0.5-0.6 g/cc
particle size distribution	:	60-80 mesh

14. A microporous hypocholesterolemising resin substantially according to Examples I - III herein.

15. A method of preparing an anionic microporous resin of hypocholesterolemising action, substantially as described in Examples I - III herein.

ERIC POTTER & CLARKSON,
5 Market Way,
Broad Street,
Reading RG1 2BN,
Berkshire,

Printed for Her Majesty's Stationery Office, by Croydon Printing Company Limited, Croydon, Surrey, 1980.
Published by The Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained.